

§Appl. No. 10/088,356
Amdt. dated February 17, 2004
Reply to Office Action of, October 14, 2003

Listing of Claims:

Please **amend** the claims as follows:

Claim 1 (Cancelled)

Claim 2 (Withdrawn)

Claim 3 (Withdrawn)

Claim 4 (Withdrawn)

Claim 5 (Withdrawn)

Claim 6 (Currently Amended) A method for identifying a virulence gene of *M. tuberculosis*, comprising ~~identifying a virulence gene of *M. marinum* bacterium according to the method of claim 1, and further comprising,~~

a) mutagenizing an *M. marinum* bacterium by introducing into the bacterium a plasmid which comprises a signature-tagged transposon, whereby the transposon integrates into and disrupts a gene in the bacterium,

b) introducing the mutagenized bacterium into a host susceptible to infection thereof,

c) identifying a bacterium which comprises a signature tagged transposon and which exhibits reduced viability in the host, compared to a non-mutagenized *M. marinum* bacterium,

d) cloning and/or sequencing a nucleic acid sequence which flanks the integrated transposon in said identified bacterium, and

e) identifying a wild type *M. marinum* gene which comprises at least a portion of said flanking sequence.

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f) comparing said flanking nucleic acid sequence to a databank of *M. tuberculosis* nucleic acid sequences, and/or comparing the sequences of peptides which are coded for by said flanking sequences to a known *M. tuberculosis* protein database, and

g) identifying an *M. tuberculosis* gene which comprises a sequence that is substantially identical to said flanking sequences.

Claim 7 (Previously Presented) A method for generating an avirulent *M. tuberculosis* bacterium, comprising mutagenizing an *M. tuberculosis* virulence gene identified by the method of claim 6.

Claim 8 (Previously Presented) An avirulent *M. tuberculosis* bacterium, produced by the method of claim 7.

Claim 9 (Previously Presented) An avirulent *M. tuberculosis* bacterium, in which one or more of genes Rv0822c, Rv3137, or Rv2348c, is mutated to render the *M. tuberculosis* bacterium less virulent.

Claim 10 (Previously Presented) An avirulent *M. tuberculosis* bacterium of claim 9, in which gene Rv0822c is mutated.

Claim 11 (Cancelled)

Claim 12 (Cancelled)

Claim 13 (Cancelled)

Claim 14 (Cancelled)

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Claim 15 (Cancelled)

Claim 16 (Cancelled)

Claim 17 (Cancelled)

Claim 18 (Cancelled)

Claim 19 (Cancelled)

Claim 20 (Cancelled)

Claim 21 (Previously Presented) An avirulent *M. tuberculosis* bacterium of claim 9, in which gene Rv3137 is mutated.

Claim 22 (Previously Presented) An avirulent *M. tuberculosis* bacterium of claim 9, in which gene Rv2348c is mutated.

Claim 23 (Cancelled)

Claim 24 (Cancelled)

Claim 25 (Previously Presented) An avirulent *M. tuberculosis* bacterium of claim 9, in which gene Rv2181, Rv1954c, Rv0987, Rv3268, or Rv2610c is mutated.

Claim 26 (Cancelled)

Claim 27 (Cancelled)

Claim 28 (Cancelled)

Claim 29 (Cancelled)

Claim 30 (Cancelled)

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Claim 31 (Cancelled)

Claim 32 (Cancelled)

Claim 33 (Cancelled)

Claim 34 (Cancelled)

Claim 35 (Cancelled)

Claim 36 (Cancelled)

Claim 37 (Cancelled)

Claim 38 (Cancelled)

Claim 39 (Cancelled)

Claim 40 (Cancelled)

Claim 41 (Withdrawn) A pharmaceutical composition, comprising an avirulent *M. marinum* bacterium of claim 5 and a pharmaceutically acceptable carrier.

Claim 42 (Withdrawn) An attenuated *M. marinum* vaccine, comprising an avirulent *M. marinum* bacterium of claim 5 and a pharmaceutically acceptable carrier.

Claim 43 (Previously Presented) A pharmaceutical composition, comprising an avirulent *M. tuberculosis* bacterium of claim 9 and a pharmaceutically acceptable carrier.

Claim 44 (Previously Presented) An attenuated *M. tuberculosis* vaccine, comprising an avirulent *M. tuberculosis* bacterium of claim 9 and a pharmaceutically acceptable carrier.

Claim 45 (Previously Presented) An attenuated *M. tuberculosis* vaccine, comprising an avirulent *M. tuberculosis* bacterium which comprises one or more mutations in one or more virulence genes identified by the method of claim 7 and a pharmaceutically acceptable carrier.

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Claim 46 (Withdrawn) A method to elicit an immune response in a fish in need of such treatment, comprising administering to said fish an avirulent *M. marinum* bacterium of claim 5.

Claim 47 (Currently Amended) A method to elicit an immune response in a patient ~~in need of such treatment~~, comprising administering to said patient an avirulent *M. tuberculosis* bacterium of claim 9.

Claim 48 (Cancelled)

Claim 49 (Cancelled)

Claim 50 (Withdrawn) A method for isolating a mutagenized *M. marinum* bacterium which exhibits reduced virulence in a host susceptible to infection thereof compared to a non-mutagenized *M. marinum* bacterium, comprising integrating a tagged transposon into the DNA of a *M. marinum* bacterium in a manner effective to produced reduced virulence, and isolating said mutagenized bacterium.

Claim 51 (Cancelled)

Claim 52 (Cancelled)

Claim 53 (Cancelled)

Claim 54 (Cancelled)

Claim 55 (Cancelled)

Claim 56 (Cancelled)

Claim 57 (Cancelled)

Claim 58 (Cancelled)

Claim 59 (Cancelled)

Claim 60 (Cancelled)

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Claim 61 (Cancelled)
Claim 62 (Cancelled)
Claim 63 (Cancelled)
Claim 64 (Cancelled)
Claim 65 (Cancelled)
Claim 66 (Cancelled)
Claim 67 (Cancelled)
Claim 68 (Cancelled)
Claim 69 (Cancelled)
Claim 70 (Cancelled)
Claim 71 (Cancelled)
Claim 72 (Cancelled)
Claim 73 (Cancelled)
Claim 74 (Cancelled)
Claim 75 (Cancelled)
Claim 76 (Cancelled)

Claim 77 (Previously Presented) An avirulent *M. marinum* bacterium, in which a gene has been disrupted adjacent to a nucleic acid of SEQ ID NOs: 4, 13, 23, 25, or 31.

Claim 78 (Previously Presented) An isolated *M. marinum* nucleic acid comprising the oligonucleotide of SEQ ID NOs: 4, 13, 23, 25, or 31, or a fragment or variant thereof; or which is complementary to, or which can hybridize under high stringency conditions to, at least a portion of said isolated nucleic acid or variant thereof.

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Claim 79 (Previously Presented) A method to identify an agent which reduces the ability of an *M. tuberculosis* bacterium to survive in a host, comprising

- a) overexpressing one of the following *M. tuberculosis* genes: Rv0822c, Rv3137 or Rv2348c in an *M. tuberculosis* bacterium,
- b) exposing said bacterium to a putative agent, and
- c) determining if the agent reduces the viability or growth of a wild type bacterium, but not the bacterium which overexpresses said gene, in a host.

Claim 80 (Previously Presented) An antibody against a polypeptide encoded by one of the following *M. tuberculosis* genes: Rv0822c, Rv3137 or Rv2348c.

Claim 81 (Previously Presented) An antibody against a peptide encoded by one of the following *M. marinum* polynucleotides: SEQ ID NOs: 4, 13, 23, 25, or 31.

Claim 82 (Previously Presented) An avirulent *M. marinum* bacterium, in which a gene comprising a nucleic acid that is at least 95% identical to SEQ ID NO: 4 is mutated.

Claim 83 (Previously Presented) An avirulent *M. marinum* bacterium of claim 82, wherein the nucleic acid comprises nucleotides 19 to 129 of SEQ ID NO: 4.

Claim 84 (Previously Presented) An avirulent *M. tuberculosis* bacterium, in which a polyketide gene is mutated to render the *M. tuberculosis* bacterium less virulent.

Claim 85 (Previously Presented) An avirulent *M. tuberculosis* bacterium of claim 84, in which the mutated polyketide gene is pks6 (Rv0405).

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Claim 86 (Previously Presented) An avirulent *M. tuberculosis* bacterium of claim 84, in which the mutated polyketide gene is pks9 (Rv1664).

Claim 87 (Previously Presented) An avirulent *M. tuberculosis* bacterium of claim 84, in which the mutated polyketide gene is pks8 (Rv1662).

Claim 88 (Previously Presented) An avirulent *M. tuberculosis* bacterium of claim 84, in which the mutated polyketide gene is pks1 (Rv2946c).

Claim 89 (Previously Presented) An avirulent *M. tuberculosis* bacterium of claim 84, wherein said polyketide gene sequence is at least 95% identical to SEQ ID NO: 8.